

**PROCEEDINGS**  
**OF THE**  
**NATIONAL ACADEMY OF SCIENCES**  
**INDIA**  
**1957**

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**VOL. XXVII**

**SECTION-B**

**PART II**

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**APRIL 1957**



**NATIONAL ACADEMY OF SCIENCES, INDIA**  
**ALLAHABAD**

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Single Copy: Rs. 5 (Inland) : 10 sh. (Foreign).

# PROCEEDINGS

OF THE

## NATIONAL ACADEMY OF SCIENCES INDIA

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Vol. XXVII

SECTION-B

Part II

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### SHORT REVIEW OF ADVANCES IN MICROBIOLOGY OVER THE PAST QUARTER-CENTURY

By

W. BROWN

*Imperial College of Science and Technology, London.*

*(Received on 26th December, 1955)*

It is a common practice on centennial (or half-or quarter-centennial) occasions to review the progress which has been made in the various fields of learning during the period which is being celebrated; and perhaps also to discuss, if somewhat venturesomely, the lines on which further advances are likely to be made. On this occasion there are some subjects—and this applies particularly to Physics—in which the advances made can truly be described as sensational; in others there may be merely a steady widening and deepening of knowledge to record, perhaps because the very nature of the subject has so determined. The field of Microbiology, in parts of which my interests have lain occupies a somewhat intermediate position: there has been, during the past 25 years, at least one advance which is entitled to the descriptive word “Sensational”, but there has been at the same time much progress along a number of lines which represents solid, if not so spectacular, achievement.

Microbiology is the science which sets out to determine the significance of microscopic organisms in the world of nature. As such, it is just about 100 years old, dating from the time the old Theory of Spontaneous Generation of microorganisms came to be abandoned among scientific workers. It was shown, not at one time and not by only one experimenter, but in researches extending over a considerable number of years, that such important natural phenomena as fermentation, putrefaction and many

diseases of plants and animals were caused by specific microorganisms (fungi, bacteria, protozoa, etc) which derived from "parents" in a manner comparable with the better-known higher plants and animals. The subject, as is usual in fields of learning, became specialised in due course into a number of branches (mycology, bacteriology and others), each with its special set of problems and techniques. It has continued to expand ever since, and at no time more rapidly than in the period now under brief review.

The sensational development naturally calls for first mention. This was the discovery of penicillin—from a fungus, *Penicillium*—which has been followed by a host of substances of similar nature and origin (Streptomycin, aureomycin, etc), though it is perhaps true to say that none of these later "antibiotics", as they are usually termed, seems to possess the same wide value for therapeutic purposes as does the first discovered member of the series. The development of penicillin as a curative agent, it should be added, has taken place only within the past 10 years though it was named and its action was demonstrated some 20 years earlier. Incidentally it is worth noting that the striking success of antibiotic therapy has reestablished the plant kingdom as a highly important source of medical aids, thus bringing about a reversal of the recent trend in the relationship of botanical science to medicine. It will be remembered that in the early days, and for many hundreds of years, Botany as a science existed largely as hand-maiden to *Materia Medica*, the chief function of botanists being to identify catalogue, and grow the plants from which certain drugs could be obtained. There came the era of chemical synthesis in the laboratory (which is by no means finished), leading to a lessening dependence on natural sources; and now there is once more a rejuvenated interest in plants (and animals) as indispensable sources of prophylactic and curative agents.

The discovery of antibiotics gave a tremendous fillip to the study of microorganisms, and especially with respect to their physiology and biochemistry. Furthermore it so happens that for various reasons, such as ease of handling and relative simplicity of organization, many microorganisms are the most convenient subjects for the study of certain fundamental physiological processes, many of them, for instance, can be grown on relatively simple and exactly reproducible media and therefore are highly suitable for studying the stages by which food substances are first broken down and then rebuilt into the complex of substances which form the body of the organism. Studies of this kind, and of course parallel ones with tissues of higher plants and animals, are now much facilitated by the use of certain modern techniques, of which two are outstanding. The first of these is the radioactive "tracer" technique whereby Elements (such as carbon) supplied in a particular food substance can be located ("traced") in definite parts of the organism and in particular substances produced by the latter. Valuable insight is thereby obtained into the nature of the chemical transformations taking place. The second technique of special value is the chromatographic method of separating substances from a complicated mixture, so that it is now possible to sort out and identify substances of biological significance to an extent which would have been quite impossible ten years ago.

Within the broad field of the physiology of organisms, micro-and macro-alike, many notable advances along special lines have been brought about within the past 25 years. There is, for example, the wide realization of the importance, indeed the necessity, of certain mineral elements for the proper growth and functioning of organisms. The essential requirement of Elements like potassium and phosphorous has long been known, but the more recent work has demonstrated that many Elements formerly thought to be of no particular significance do in fact play a very important part.



Here again, and for the reasons already stated, micro-organisms have proved to be of great value as experimental subjects. A striking feature of such Elements (e.g. copper, zinc, molybdenum, boron) is that, though they may be essential for the growth of the organism, the merest trace is often sufficient—whence they are often spoken of as “minor Elements”—and in fact a quantity greater than a mere trace is liable to be deleterious. Deficiency in respect of these elements usually leads to disordered growth of the organism—to a so called “physiological disease”—and numerous examples of this kind have been elucidated in recent years among economically important crop plants. Furthermore there are remarkable instances of forage crops which, when grown on land that is inadequately supplied with a particular minor Element, do not in themselves show any abnormal effect; yet, when they are fed to animals, the latter may develop a serious deficiency disorder. The importance of this type of work to man and his animals needs no stressing.

Mention of minor elements leads directly to the consideration of enzymes, inasmuch as some at least of the latter are known to be activated by the presence of a trace of a particular element. The subject of enzymology has, in recent years, swollen to portentous dimensions; it would be no exaggeration to say that it has entered upon a new and throbbing phase of its development. Its foundations were laid in the latter part of the 19th century, then followed some decades of relative quiescence and now it is again in a period of intense activity. This is not surprising when one remembers that enzymes are substances intimately associated with life—they are invariably present in living organisms and conversely are not known to occur elsewhere than in living organisms. Hence the modern view that the vast majority, and it may be the totality, of vital reactions are brought about by the agency of enzymes systems. Such a view, it may be added, would go a long way towards explaining what has always been a profound enigma, viz, that certain chemical transformations which are very difficult to bring about in the laboratory appear to proceed quickly and easily in the living organism.

The fundamental importance of enzymic studies is therefore unquestionable. But it is clear that the subject is one of very great complexity. The interconnection between enzymes and mineral elements has already been mentioned; there is also evidence that the functioning of enzymes is bound up with the presence of specific organic substances—variously known as hormones, vitamins, auxins etc. according to the type of reaction with which they are associated. The unravelling of this complex may require the labours of generations of investigators and one can venture to prophesy that there will be much research in this field during the next 25 years period of the life of this Academy. Within the broad field which has just been indicated microbiologists will, without doubt, play a highly significant part.

A development of peculiar interest, which has largely taken place within the period under review, has been the application of cytogenetical principles and methods to the study of microorganisms. For long it was believed that the latter were unsuitable for that type of study: their nuclei, and therefore their chromosomes, were as a rule inconveniently small for microscopic observation and the technique of artificial crossing, which is a key process in genetical research and which is as easily carried out with most higher plants, was more difficult with fungi and still more so with bacteria. This difficulty has now been overcome for a wide range of these organisms, with results that are of great interest, both practically and theoretically. One practical outcome is that it is now possible to breed new strains of microorganisms which possess specific desirable properties, for example new strains of *PENICILLIUM* which are specially active in the secretion of penicillin, new yeasts and other fungi for use in

the fermentation industries, and so on. From the theoretical point of view great value attaches to the production of new strains—by intercrossing or by induced mutation or by a combination of both methods—which show certain slight modifications of biochemical activity. By the use of such an array of organisms, much light has been thrown and is being thrown on the sequence of events taking place in various fundamental biochemical processes. In other words they furnish a new tool for the biochemist in studying the activities of organisms.

Within the limit of this brief review it is hardly possible to do more than mention some highly important, though less generalized, fields of microbiological investigation. The relation of bacteria (and to a less extent, of fungi) to animal diseases is a theme for medical and veterinary study, but that of fungi (and to a less extent, of bacteria) to diseases of plants is strictly a botanical and agricultural subject. The damage caused by fungal and bacterial parasites to the crop plants of man, and therefore to his domestic animals and so finally to himself, has always been considerable; it is important at the present day and one can foresee that, by the natural growth of the world's population, the seriousness of this problem is likely to increase. The protection of crop plants, both in the field and in the store, is an urgent requirement in all countries and the urgency is likely to become greater with the passage of years. Hence the large amount of research which has been and continues to be devoted to the discovery of more effective chemical agents for the purpose and to the devising of better methods of applying them to the crops. An alternative method of achieving the same desirable result is to breed races of crop plants which combine high yielding power and quality with resistance to important parasites, and some very notable successes have been gained by this means. Researches on the factors determining soil fertility—which, incidentally, has important microbiological components—have the same ultimate object, viz. to improve the soil as a medium for the growing of crop plants.

Problems of the kind here outlined may be of local importance only and therefore their solution is primarily the responsibility of the country concerned. Many of them, however, with perhaps some modification from place to place, are wide-spread and call for investigation on an international scale. During the past 25 years one can point to much international cooperation in the task of combating plant diseases; we all hope and expect, I am sure, that in the like period which is before us a similar, or even greater, degree of cooperation will be forthcoming.

# A NEW HORIZON IN SOIL MICROBIOLOGY\*

By

John N. Couch

Keenan Professor of Botany, University of North Carolina, U. S. A.

(Received on December 27, 1955.)

The discovery of antibiotics from soil-inhabiting actinomycetes has catapulted this group of humble organisms into first rank importance. In laboratories all over the world microbiologists are searching the soil for new actinomycetes in the hope of finding more antibiotics. This has resulted in great interest in the taxonomy and physiology of this group.

My interest in the actinomycetes arose from a purely accidental discovery. It was while examining lower fungi in soils sent me from the Philippines that the remarkable genus *Actinoplanes* was found. When first seen, the organism was growing on a piece of boiled grass along with some large chytrids and was assumed to be a small chytrid perhaps belonging in the genus *Rhizidium*. It has a very delicate mycelium suggesting that of certain chytrids and formed sporangia which produced vast numbers of very minute swimming spores. Further study showed that the zoospores when mature were usually arranged in a coil within the sporangium, suggesting the coils of conidia in certain species of *Streptomyces*. Furthermore, dark field observations, staining, and photographs with the electron microscope, showed that the flagella on the motile spores were entirely unlike any yet described in the fungi, but strikingly resembled those of certain bacteria. Other isolations were made and it soon became apparent that we had a group of organisms which resembled the actinomycetes but differed in having sporangia and motile spores. Because of this unique character the new genus was named *Actinoplanes*, i.e. the actinomycete with plano-spores.

It is remarkable that *Actinoplanes* which is very common in soils, had not been discovered before among the vast number of isolates of *Streptomyces*. The explanation lies in the fact that, in isolating by the soil-dilution-in-agar methods, *Streptomyces* grows more rapidly and soon overgrows *Actinoplanes*, which is a slow grower. I have isolated several species using Jensen's dextrose casein agar, but better results have been obtained with three per cent agar in water, since *Streptomyces* and molds grow less vigorously on the plain agar. However, the most rapid and best are modifications of the chytrid-isolating methods.

In this procedure a level teaspoonful or less of soil is put in a sterile Petri dish and flooded with sterile water. The dirt is heaped up on one side of the dish and when it has settled, a few pieces of boiled *Paspalum* grass leaves are added in such a way that

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\* The work on which this paper is based was made possible by a grant from the National Science Foundation. I am grateful to Dr. E. K. Goldie-Smith for help and for Mr. W. J. Koch's help in studying the motile cells.

the leaf is only partly submerged. After about seven days the leaves are examined with a binocular dissecting microscope ( $\times$  about 100), under a strong direct light which passes first through a flat glass dish containing water to filter out the heat. If *Actinoplanes* is present, it can be recognized by the white glistening sporangia formed in the air at the surface of the water. A small piece of the leaf with sporangia is cut out and transferred to three per cent plain agar and then, with very fine needles, a single sporangium is dissected out from the mass. Bacteria which adhere to the surface can usually be removed by pushing or dragging the sporangium over the surface of the agar for a distance of two to three centimeters. The single sporangium is then cut out with a small block of agar and transferred to a test tube containing Czapek's or other suitable agar and in this way pure cultures are made. In baiting for *Actinoplanes*, pollen of *Liquidambar* is even better than *Paspalum* leaves. The pollen is dusted lightly on the water and usually remains floating at or near the surface. *Actinoplanes*, if present, appears on the pollen in three to four days. Under the binocular dissecting microscope, it is easy to pick up a pollen grain bearing *Actinoplanes*, transfer it to plain agar and proceed as above for making a single sporangial culture. The pollen grain technique has proven so simple and easy that our stock of isolates has increased to about 180.

Our first isolates of *Actinoplanes* were from tropical soils, but further studies have shown it to be worldwide in distribution. We have not yet made any well-planned studies of its distribution and abundance in various soil types but the results so far indicate that *Actinoplanes* is very abundant in soils rich in humus and is rare or absent from clay banks and other soil habitats with little or no humus.

While some species of *Actinoplanes* will grow on such animal sub-strata as hair, horn, hoof and feathers, most prefer such plant material as boiled leaves and slices of stems, roots, tubers and seeds of various vegetables and grasses. The most useful substrates so far found are boiled *Paspalum* grass and air-dried pollen of *Liquidambar styraciflua*.

Ready recognition of *Actinoplanes* on *Paspalum* leaves depends on the formation of large numbers of sporangia. The sporangia on leaves are initiated and formed in the air on or above the surface of the water. Under a binocular dissecting microscope the sporangia appear as globose to irregular, rounded, white, glistening, minute bodies, which may be formed in clusters, or a continuous sheet, or scattered over the surface of the leaf. The irregular sporangia of some species strikingly resemble minute grains of sand and could easily be passed over for such. The mycelium on grass in water is usually within the leaf and forms a submerged fringe around its edge. In some species a considerable number of aerial threads are formed, and in a few, such threads are abundant. The mycelium is colorless, or pinkish to pale orange, much branched, sparingly septate and composed of very fine threads,  $0.5-1.5 \mu$  in diameter, much as in *Streptomyces* or *Micromonospora*.

On *Liquidambar* pollen the mycelium penetrates throughout the grain and extends out into the water a distance about equal to the diameter of the pollen grain. Thicker threads grow up to and above the water surface and there form in the air the sporangia which show up so beautifully as white glistening bodies when brightly illuminated under the binocular dissecting microscope.

Cultures on leaves and pollen are useful because the sporangia are readily formed on these and in a given species vary less in size and shape than they do on artificial and synthetic media. Furthermore, it seems likely that pollen is an important natural

food for *Actinoplanes* growing in the soil, and thus, cultures on pollen give us a means of determining what *Actinoplanes* looks like in its natural habitat.

In order to compare *Actinoplanes* with its nearest relatives *Micromonospora*, *Streptomyces* and *Nocardia*, it has been necessary to grow the isolates of *Actinoplanes* on the same culture media that have been used in growing these three genera, i.e. various agars, gelatin, and nutrient broths. A large number of the three genera mentioned were obtained for comparative study.

Some species of *Actinoplanes* on certain agars strikingly resemble *Micromonospora* in color and in general habit of growth. The structure of the vegetative mycelium of the two is indistinguishable. *Micromonospora*, in general, grows more slowly than *Actinoplanes* and on certain agars turns greenish black, as the microspores are formed, unlike *Actinoplanes*. The two may easily be distinguished by their reproductive characters. *Micromonospora* forms its characteristic spores singly or in grape-like clusters but never in chains. The conidia of *Actinoplanes* are formed singly and also in chains. Furthermore, none of the collections of *Micromonospora* forms sporangia.

Some of the species of *Nocardia* strikingly resemble some of *Actinoplanes* in color and in gross morphology. Several species of *Actinoplanes*, when grown on certain agars, potato dextrose for example, form a small pasty growth which when mounted and crushed under a coverslip, separates into small irregular segments, much as in *Nocardia*. Such growth, however, is not the normal condition for any species of *Actinoplanes*. In general, the species of *Actinoplanes* are more vigorous growers, more brilliantly colored and do not show the paste-like growth of *Nocardia*. None of the species of *Nocardia* formed sporangia when grown under the conditions most favorable for sporangial formation.

*Streptomyces*, in general, grows more vigorously than most species of *Actinoplanes* and species of the former, when first isolated, produce their characteristic conidia in coiled or straight chains on aerial conidiophores. The similarities and differences in growth and reproduction of the two genera are beautifully shown in cultures on pollen when the two are found together in the original dish. Both have much the same vegetative mycelium but the numerous aerial conidiophores of *Streptomyces* extend upward from the pollen 0.5—1mm, while the sporangia of *Actinoplanes* barely clear the water with their relatively short stalks. On Czapek, peptone-Czapek and potato dextrose agars, *Streptomyces* is usually more vigorous grower than *Actinoplanes*, but the latter is usually more brilliantly pigmented. *Streptomyces* typically has aerial hyphae, which most species of *Actinoplanes* lack. The most striking difference between the two genera lies in the fact that *Actinoplanes* reproduces by the formation of sporangia in which are produced motile spores, while *Streptomyces* forms conidia on conidiophores. This difference, while perhaps enough to justify a new family for *Actinoplanes*, is not so sharp as it may seem. In some species of *Actinoplanes* the spores when mature, are arranged in a continuous coil within the sporangium and in other species coils are formed which break up into conidia. In some species of *Actinoplanes*, the conidia become motile. It is likely that *Actinoplanes* is closer to *Streptomyces* than to either *Nocardia* or *Micromonospora*. Indeed we seem to have the connection between those two genera in the recently described genus *Streptosporangium*. This genus forms conspicuous aerial hyphae on many agars and in the type species the aerial hyphae are usually arranged in concentric circles. It is characterized by having sporangia with non-motile spores and, in the type species, in addition to the sporangia, conidia are formed in bent or coiled chains.

Several species of *Actinoplanes* produce, under certain conditions, a very distinctive musty odor, given off only by cultures which are forming sporangia in large quantities. Similar odors have been described for some of the aerobic species of *Streptomyces*. I have not detected any distinctive odors for *Nocardia* or *Micromonospora*.

By using the staining technics of Robinow and DeLamater it has been shown that the nuclear bodies in *Actinoplanes* are like those described by Klieneberger-Nobel for *Streptomyces*, which in turn are like the nuclear bodies described by Robinow and others in true bacteria.

The most distinctive features of *Actinoplanes* are the sporangia and motile spores. As already pointed out, the sporangia on leaves and pollen grains in water are formed in the air just at or above the surface of the water. If the substrate is at the surface, the sporangia may be sessile or on branched or unbranched stalks elevating them above the surface. In many species, when cultured on certain agars, the sporangia are formed at the surface in the air on vertical, unbranched hyphae which we have called palisade hyphae; in others the sporangia are borne on wavy hyphae; in a few species, the sporangia are formed on branched or unbranched stalks which are elevated above the agar surface. When abundant, the sporangia give the culture a fine powdery appearance.

The sporangia vary greatly in size and shape in the different species but are fairly constant in each species on any particular culture medium. In size they vary from about 5–35  $\mu$  in diameter and the common shapes are spherical, cylindrical, irregularly rounded, and lobulate.

If mature sporangia are submerged in water, dehiscence begins after a few minutes to a couple of hours. In some species, the spores emerge through a small hole in the sporangial wall and in others the wall may crack irregularly. The spores are exceedingly minute, 1–1.5  $\mu$  in diameter, and under low power appear to be round but under higher magnifications are mostly subglobose, angular or slightly pyriform. The spores are exceedingly vigorous swimmers, but no flagella can be seen with certainty by bright field, by phase contrast or by the usual dark field illumination. Furthermore, the technic (osmic acid, crystal violet) so successful in staining the flagella of fungi and algae is ineffective here. Spores of several species of *Actinoplanes* have been stained by the usual bacterial stains and when so treated are found to have a tuft of polar flagella. This observation has been corroborated by numerous electron micrographs. This tuft of flagellar material on the dead spores consists of thirty to forty minute strands. If the living spores mounted in a solution of methocel are observed under dark field with a carbon arc lamp, a single flagellum appears to originate from the front end of the spore and to be directed backwards as the spore swims.

Our studies of *Actinoplanes* indicate that this new genus belongs in the Actinomycetes. But what bearing has the discovery of sporangia and motile spores on the relationships of the entire order? For over fifty years, it has been recognized that the structure of the motile cells in algae, fungi and protozoa is of prime importance in determining relationships. In recent years, Manton and her associates in a series of brilliant papers have made the very important discovery that the flagella of fungi, of green, yellow-green, and brown algae, and of mosses and ferns will dissociate into eleven strands when the cells begin to die. This remarkable behavior of the flagellum is a peculiarity of the motile cells of plants and animals, and so far as known, does not occur in any bacterium, nor does it occur in *Actinoplanes*. This would seem to indicate that, so far as the structure of the motile cells is concerned, *Actinoplanes* is

rather sharply separated from the fungi and is phylogenetically connected with the true bacteria. We cannot ignore, however, the striking resemblance of the mycelium and the sporangia of *Actinoplanes* to those of the chytrids, and future studies may show that *Actinoplanes* represents a connection between bacteria and the lower fungi.

The discovery of sporangia and zoospores in the actinomycetes is of great interest particularly in the opening up of entirely new concepts of the phylogeny of the order. Furthermore, these organisms, which are very abundant, are doubtless of importance in soils in the formation of humus. A good many of the species are active antibiologically and may find uses in medicine or agriculture. Thus an accidental discovery has opened up a whole new field for investigation by the soil microbiologists.

# STUDIES ON THE CUTICLE OF SOME INDIAN SCORPIONS\* : SULPHUR LINKAGE AND PURINES

By

S. C. SHRIVASTAVA

Zoological Laboratory, University of Lucknow

(Received on 1st August 1957)

## Summary

The present studies have revealed the absence of —S—S—bonds and purines in the cuticle of the scorpions *Palamnaeus bengalensis* and *Buthus tamulus gangeticus*. These scorpions, therefore, differ in this respect from the scorpion *Palamneus swammerdami* where purines and —S—S— bonds have been reported (Krishnan 1953:1954) to play an important role in the hardening of the cuticle.

## Introduction :

Role of —S—S— bonds similar to the phenomenon of keratinization was described by Lafon (1943) in the hardening of cuticle of *Limulus*. Krishnan (1953) has also emphasised this phenomenon in the hardening of the cuticle of the scorpion *Palamneus swammerdami* where he has not only described the hardening of the untanned epicuticle by —S—S— bonds but has also attributed the bisulphide linkage with the tanning process of the exocuticle. It was thought desirable to examine the local scorpions also in this light.

## Material and Method :

The scorpions *Palamnaeus bengalensis* and *Buthus tamulus gangeticus* commonly found at Lucknow were used for the present study. Methods employed have been discussed in the text.

## Observations and Results :

### A. Bisulphide linkage

The present author performed a number of experiments to test sulphur in the cuticle of *Palamnaeus bengalensis* and *Buthus tamulus gangeticus*. On treating the transverse sections of the cuticle with alkaline solution of Sodium sulphide (Brown 1950) there was no indication of softening, swelling or disruption in any layer of the cuticle even when the treatment was prolonged considerably. The exocuticle did not lose its amber colour. The cuticle also did not show reversal of the staining reaction of Mallory.

\*Part of Ph. D. thesis.



When the cuticle of *Palamnaeus bengalensis* and *Buthus tamulus gangeticus* was treated with thioglycollate (Trim 1941) it did not swell. On treating hand section of the cuticle of *Palamnaeus bengalensis* and *Buthus tamulus gangeticus* with alkaline lead acetate solution no black precipitate was obtained.

Parallel controls were maintained, while performing these reactions, using pieces and sections of human finger nail which is known to contain —S—S— bonds. Excellent results were obtained in the controls with the same reagents.

When pieces of clean cuticle of *Palamnaeus bengalensis* and *Buthus tamulus gangeticus* were treated in 40% potassium hydroxide for some time and a few drops of sodium plumbate solution were added to it no black precipitate was produced, though it was well produced in the control (finger nail). Thus it would appear that —S—S— bonds are altogether lacking in the cuticle of *Palamnaeus bengalensis* and *Buthus tamulus gangeticus*.

Further evidence in support of this conclusion was obtained when a few pieces of cuticle were fused with a mixture of sodium carbonate and potassium nitrate in proportion of 2:1 until the formation of a colourless mass (Hawk, Oser etc. 1949). This was cooled and the cake dissolved in a little warm water, filtered and acidified with hydrochloric acid. On addition of barium chloride to this solution at boiling point, a white precipitate was not formed with this experimental substrate, although a white precipitate did appear in the control (finger nail). It is, therefore, quite evident that sulphur is absent in the cuticle of the scorpions *Palamnaeus bengalensis* and *Buthus tamulus gangeticus*.

#### B. Purines

Pieces and hand sections of the cuticle of *Palamnaeus bengalensis* and *Buthus tamulus gangeticus* were kept in 2 to 3 drops of concentrated nitric acid and the fluid carefully evaporated on a water bath. A purplish red colour was not produced on cooling and addition of ammonium hydroxide. This shows that purines are not present in the cuticle of these scorpions. Tests were also performed on the cuticle of juvenile *Palamnaeus bengalensis* and *Buthus tamulus gangeticus* but these also yielded negative results.

#### Discussion :—

In *Palamneus swammerdami* the cuticle is described to get swelled when treated with sodium thioglycollate solution and blackened with alkaline lead acetate solution indicating the presence of sulphur (Krishnan 1953). Treatment with alkaline sodium sulphide solution is described to render the cuticle soft, swollen, disrupted and more susceptible to the action of acids. After the treatment, the cuticle shows reversal of staining reactions of Mallory in the same way as noticed in a tanned cuticle after Diaphanol treatment. The sodium sulphide solution affects the —S—S— bonds and renders the protein soluble (Brown 1950). As the tanned exocuticle of *Palamneus swammerdami* lost markedly its amber colouration. Krishnan (1953) attributed a close association of the bisulphide linkage to the tanning process in the exocuticle. Krishnan (1953) also reported that the epicuticle of *Palamneus swammerdami* which is untanned is also hardened by —S—S— bonds as reactions similar to those for the exocuticle were seen for the epicuticle. In *Palamnaeus bengalensis* and *Buthus tamulus gangeticus*, as proved in the preceding pages, none of

the above reactions could give positive results in any layer of the cuticle although results similar to those of *Palamneus swammerdami* were obtained in pieces of human finger nails used as controls.

Krishnan (1954) also found purines related to the regions of the cuticle of *Palamneus swammerdami* which are hardened by —S—S— bondings and attributed it a role similar to as played by urea in the transformation of serum albumin and globulin into a firm transparent gel (Haggins 1951). In the presence of urea a consecutive process is described to occur in which the free —SH—group of one albumin molecule reacts with a —SH—group in a neighbouring molecule to form an intermolecular disulphide bond. Krishnan also found that purines were more concentrated in the juvenile stages of *Palamneus swammerdami*. It has already been shown in the preceding pages that purines were not found in both adult and juvenile *Palamneus bengalensis* and *Buthus tamulus gangeticus*.

The results obtained by the author in *Palamneus bengalensis* and *Buthus tamulus gangeticus* are compared with those of *Palamneus swammerdami* (Krishnan 1953 and 1954) in the table.

TABLE

Summary of histochemical reactions of sulphur and purines in the cuticle of *Palamneus swammerdami* (Krishnan 1953 & 1954), *Palamneus bengalensis* & *Buthus tamulus gangeticus*.

Reactions	<i>Palamneus swammerdami</i> (Krishnan 1953 & 1954)			<i>Palamneus bengalensis</i> & <i>Buthus tamulus gangeticus</i>		
	Epi- cuticle	Exo- cuticle	Endo- cuticle	Epi- cuticle	Exo- cuticle	Endo- cuticle
(1) Alkaline Lead acetate (blackening)	+++	+++	—	—	—	—
(2) Sodium sulphide a. (solubility in) b. (Mallory)	++ red. (unstained pre- viously)	+++ dark red. (unresponsive previously)	+	— no change	— no change	— no change
(3) Thioglycollate (swelling in)	++	+	—	—	—	—
(4) Murexide test	++	—	—	—	—	—

The present study has, therefore, revealed that —S—S— bonds are altogether absent and therefore can not be involved either in the hardening or phenolic tanning of the cuticle of the scorpions *Palamneus bengalensis* and *Buthus tamulus gangeticus*. This shows that hardening involving bisulphide linkage may not be a universal process in scorpions.

#### Acknowledgments :

The author is thankful to the Dept. of Zoology, University of Lucknow for the research facilities provided to him. He is also deeply indebted to Prof. M.B. Lal under whose guidance the present studies were carried out.

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# EFFECT OF DIFFERENT CARBON COMPOUNDS ON THE GROWTH AND SPORULATION OF *FUSARIUM COERULEUM* (LIB.) SACC.

By

R. N. TANDON AND GANGA PRASAD AGARWAL

*Department of Botany, University of Allahabad.*

*(Received on 17th April, 1957)*

Carbon is essential for all living organisms and it plays an important part in the nutrition of the fungi. In fact the work of earlier investigators on the physiology of fungi was mainly on Carbon requirements of different organisms.

A survey of the literature shows that no single sugar can be considered to be the best source of carbon for all micro-organisms. There is no doubt that most fungi can use a large variety of carbon sources for their growth and reproduction. The numerous investigations on carbon nutrition of fungi clearly indicate the importance of this problem. Carbon constitutes about half the total dry weight of a fungus. In view of the importance of different carbohydrates on the growth and sporulation of various fungi—specially when the behaviour of different strains of a single species may be different—it was considered desirable to study the carbon requirements of two strains of *Fusarium coeruleum* isolated from potato and 'Arvi' (*Colocasia antiquorum*).

## MATERIALS AND METHODS

Asthana and Hawker's medium 'A' containing 5 gm. glucose, 1.75 gm.  $\text{KH}_2\text{PO}_4$ , 0.75 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 3.5 gm.  $\text{KNO}_3$  and one litre water was used as the basal medium. In order to study the effect of various carbon compounds they were singly substituted for glucose of the basal medium. The quantity of different compounds was so adjusted as to contain an amount of carbon equivalent to that present in 5gm. of glucose except for starch, which was added in the same quantity as glucose. The following compounds were used:

### 1. Carbohydrates.

#### *Monosaccharides*—

a. Hexoses—glucose, galactose, mannose.

b. Pentoses —Arabinose, rhamnose.

*Disaccharides*—Lactose, maltose, sucrose.

*Trisaccharides*—Raffinose.

*Polysaccharides*—Starch.

## II. Alcohols-Dulcitol and Mannitol.

Throughout the experiment only guaranteed reagents, pyrex glass wares and double distilled water were used. Liquid cultures containing 50 c.c. of the medium were taken in 150 c.c. conical flasks. Four replicates were used for each treatment.

The solutions were autoclaved at 15 lbs. pressure for 15 minutes and after inoculation they were incubated at a temperature of  $23^{\circ}\text{C}+1^{\circ}\text{C}$ . for 15 days.

### OBSERVATIONS

The dry weights of the two strains are recorded in Table 1.

The sporulation of both the strains was more or less similar and was good on all the media except on dulcitol and mannitol where it was fair only.

TABLE 1.

*Showing the dry weights of the two strains of Fusarium coeruleum on media containing equivalent quantities of different carbon compounds*

C-compounds	Dry weight in mg.	
	<i>Fusarium coeruleum</i> from potato	<i>Fusarium coeruleum</i> from <i>Colocasia antiquorum</i>
Glucose	93.0	140.6
Galactose	113.6	150.4
Mannose	90.4	140.0
Arabinose	76.6	120.4
Rhamnose	70.0	117.6
Lactose	88.0	137.2
Maltose	88.0	133.0
Sucrose	87.4	136.8
Raffinose	86.2	123.8
Starch	105.2	147.2
Dulcitol	56.8	100.0
Mannitol	55.4	96.2
No carbon compound	0.0	0.0

As reported by Tandon and Agarwal (1953) the growth of *Fusarium coeruleum* isolated from potato was poorer than that of colocasia strain. The above tale clearly shows that the two strains of *Fusarium* could utilize all the carbon compounds used in the present investigation. It was also evident that the best growth of both the organisms was on galactose. Dulcitol and mannitol were found to be the worst sources of carbon. Other substances supported good or satisfactory growth. Both of them could not grow in complete absence of any carbon compound.

The various carbon compounds could be arranged in the following order :—

Galactose, starch, glucose, mannose, lactose, maltose, sucrose, raffinose, arabinose, rhamnose, dulcitol and mannitol.

The colocasia strain showed only one minor difference as the growth was slightly better on sucrose than on maltose.

In general the sporulation was profuse and it was not possible to estimate minor variations on different media. The sporulation was, however, much less on media containing dulcitol or mannitol.

### CONCLUSIONS AND DISCUSSIONS

Glucose is generally used as a carbon source for most of the fungi but a few of them are unable to utilize it, or any other sugar as a source of carbon. Schade (1940) and Thimann (1940) reported that *Leptomitus lacteus* was unable to utilize glucose, fructose, galactose or sucrose. Weimer and Harter (1921) and Tochinal (1926) investigated *Sphaeronema fimbriatum* and *Fusarium lini* respectively and they found that those organisms were incapable of utilizing glucose. The two strains of *Fusarium coeruleum* could grow well on glucose and in this respect they behaved like the fungi investigated by Leonian (1924), Steinberg (1939), Saksena et al (1949), Tandon (1950), Wolf (1953) and others. Galactose, though reported to be poor source of carbon by Horr (1936) and Brocks (1951), was found to be most suitable for the two strains of *Fusarium coeruleum*. Mosher et al. (1936) Wolf et al. (1950) Srivastava (1951) and others have also reported it to be good source of carbon for the fungi investigated by them. Gordon (1950) and Fergus (1952) reported Mannose as a good source of carbon while Ezekiel et al. (1934) and Horr (1936) found it unsuitable but in the present investigation mannose proved to be a good source of carbon though it was not so satisfactory as galactose, starch or glucose. Wolf et al. (1950), and Wolf (1953) found that pentoses (arabinose and rhamnose) were poor sources of carbon but the two strains of *Fusarium coeruleum* had moderate growth on those two substances.

La Fuje (1937) and Steinberg (1939) found their fungi to be incapable of utilizing lactose. Schade (1940) and Tochinal (1926) reported that maltose and sucrose were poor sources of carbon. Blank and Talley (1941) and Wolf et al. (1953) found lactose, maltose and sucrose as good sources of carbon. Kinsel (1937) and Stevens and Larsh (1939) reported that *Diplodia macrospora* could grow only on disaccharides and not on media containing glucose or other monosaccharides. In the present investigation the utilization of monosaccharides and disaccharides (lactose, maltose and sucrose) by the two organisms was almost similar.

Saksena and Mehrotra (1949) as well as Srivastava (1951) observed that raffinose was a poor source of carbon but this trisaccharide was found to support good growth of the present fungi and in this respects the results were similar to those of Wolf (1953).

Wolf (1953) reported that *Ustilago zaeae* could not utilize starch. Fergus (1952) obtained poor growth of *Penicillium digitatum* on starch while Brocks (1951), Srivastava (1951), and Wolf, et al. (1950) found it to be a good source of carbon. In the present investigation also starch was able to support good growth. According to Lilly and Barnett (1951) only those fungi which produce amylase are able to utilize starch and this ability is common among fungi but is not universal.

Hawker (1939) as well as Saksena and Mehrotra (1949) reported that mannitol and dulcitol were poor sources of carbon. Wolf et al (1950, and Patel et al. (1950) found that these alcohols were good for the fungi investigated by them. They were, however, not satisfactory for the good growth of the two organisms under investigation.

It has been reported by many investigators that sporulation of fungi may be influenced by the kind of carbon compounds available for their growth. Mathur et al. (1950) reported good sporulation of *Colletotrichum lindemuthianum* on galactose and glucose and poor on mannose and mannitol. Patel et al. (1950) reported sucrose as a good source of carbon for the sporulation of many fungi. In the present investigation sporulation of the two fungi was found to be good on all the carbon sources tried except on dulcitol and mannitol which supported fair sporulation only.

The authors have also observed that the growth increased with the increase in the amount of carbohydrates. Fresh potatoes contain 19.1% carbohydrates out of which 14.7% is starch which is very suitable for *F. coeruleum* and this may account for heavy losses. It appears that whatever carbohydrates may be present in potato and 'Arvi' the fungus will grow more vigorously when larger quantities become available. This may explain the greater susceptibility observed by Pethybridge and Lafferty (1917) when the maturation of the tubers advances.

#### SUMMARY

The growth and sporulation of the two strains of *Fusarium coeruleum* isolated from potato and 'Arvi' (*Colocasia antiquorum*) on different sources of carbon has been studied. It was found that the two strains were not very specific in the utilization of different carbon compounds. The best growth of both the strains was on galactose. Dulcitol and mannitol were found to be the poorest sources of carbon. The rest of the carbohydrates supported good or moderate growth. Carbon was found to be essential for the growth of the two organisms.

Sporulation was good on all the carbohydrates but it was much less on dulcitol and mannitol which supported fair sporulation only. It was evident that every source of carbon normally present in potato and 'Arvi' could be suitable for the dry rot causing organisms.

#### ACKNOWLEDGMENTS

The authors are grateful to Professor Shri Ranjan M. Sc. (Cantab), D. Sc. (State-France), Head of the Botany Department, University of Allahabad for providing the laboratory facilities.

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# ON THE PARENTAL CARE OF THE EUROPEAN EARWIG, *FORFICULA AURICULARIA* LINN: (DERMAPTERA)

By

BASANTA KUMAR BEHURA

*Department of Zoology, Ravenshaw College, Cuttack.*

*Received on December 15th, 1956.*

(Communicated by Dr. U. S. Srivastava)

## INTRODUCTION

The most interesting feature in the biology of the common European earwig, *Forficula auricularia* Linn. is the parental care shown by the female for her eggs and young. The trait was first observed by the Swedish naturalist Carl de Geer (1720-78) in 1758-59 and since then much about the natural instinct of *F. auricularia* have gained access to the entomological literature. Of recent works on the subject mention may be made of the observations of Fulton (1924), Goe (1952) and Crumb, Eide and Bonn (1941) in the U. S. A. and of Weyrauch (1929) in Germany. In the British Isles, mention may be made of the observations of Brindley (1914), Chapman (1917), Worthington (1926) and Lucas (1932). There has been disagreement in the observations of these workers and personal observations by British writers on the domestic habits of the earwig is greatly lacking (Burr, 1939). This prompted the author to give special attention to the subject while studying the life-history of *F. auricularia* at the Department of Zoology of the University of Edinburgh (Behura, 1950, 1950a, 1956) during 1948 and 1949.

During my studies on the parental care, I unwittingly in Edinburgh made the same observations as Fulton (1924) in Oregon and to some extent as Worthington (1926) in England. Hence this paper deals only with my personal observations on the points over which discrepancies occur. As for example, Camerano (1880), Brindley (1914) and Lucas (1932) opine that the mother ceases to care for her young after hatching, whereas, De Geer (1773), Fulton (1924) and Crumb *et al* (1914) hold the opposite view.

## MATERNAL CARE OF THE EGGS

The female earwig takes care of her eggs with the greatest devotion. Eggs deprived of this care are almost invariably mildewed. In my investigations, unless in highly developed condition and about to hatch in two or three day's time, eggs in no case hatched in the absence of the female, probably because it is necessary for them to be kept in a moist condition, and yet protected from mould. The care of the eggs includes licking, turning and frequent shifting of their position. In carrying the eggs,

the female opens the mandibles and holds the egg between them by means of palpi. It may be mentioned here that Brindley (1914) and Lucas (1920) erred in their statements that the eggs are collected by means of the first pair of legs. The last author (Lucas, 1932) however corrected himself in stating that the female earwig lifted the eggs with her jaws.

If a female earwig with her egg mass is taken from the soil and placed in a suitable container in which eggs have been mixed with a quantity of soil, she at once sets about retrieving the eggs. The eggs are collected into little provisional heaps and then assembled into one. While collecting eggs and their mothers in the field, I used to put a single female and her eggs in a small glass vial with soil and leave them overnight to transfer them to a suitable larger container the next day. Invariably I found that the female earwig collected her eggs and would be lying over them in a nest which she had made at the bottom of the glass vial during the night.

Although it is instinctive in the female earwig to make a nest, the author has not come across any reference in the literature to a male earwig carving out a nest. On the contrary Fulton (1924) stated that the males never attempted to dig cavities for themselves, but took advantage of existing crevices, no matter how poor the accommodations. It is interesting to note that in one instance, a male earwig which had been collected in the field in April, 1948 and left overnight in a glass vial with soil was discovered the next morning to have carved out a cell for itself in the same.

The female earwig lies over her eggs in the same way as a hen broods over her eggs. On March 3, 1948 I had collected a male and a female earwig in the field and left them together in a glass vial in the laboratory. On March 15, I discovered that a nest had been made at the bottom of the glass vial in which the female earwig was brooding over a cluster of eggs. I turned the glass vial upside down on a petri dish. The female hurriedly caught hold of an egg between her mandibles, rushed into the heap of loose soil, tucked the egg underside her and lay over it. I put the eggs and the couple in a big container. No sooner were they released into the glass vial the male seized an egg hurriedly between his mandibles and devoured it.

In my laboratory cultures, in many bottles too moist conditions encouraged the growth of fungus. The female always shifted the eggs to the side of the container where there were no fungus growing. Even one was found shifting eggs onto a small pebble placed for her hiding. Where the bottom was too moist, they would remove their eggs to the vertical sloping wall of the glass vial and would lie vertically putting their anterior part of the body immediately below the egg mass so as to give them support, and the antennae moving over them so that the eggs may not fall into the standing water. This was doubtless a desperate attempt to make the best of a bad situation. However, this behaviour exhibited a remarkable instance of maternal solicitude.

Not only the female lies over her eggs but also guards them against the intruders. At the time of oviposition she expels the male out. In the laboratory, the male is found high up in the glass vial trying to remain as far away from the female as possible; in the field the male is usually found just above the nest. In the laboratory, there is some indication, that in some cases, the female seems to tolerate the presence of the male sometime after oviposition, an observation which is in agreement with that of Goe (1925) made in U. S. A. During the period of brooding, the female is ferocious and would attack anyone disturbing her. In my laboratory cultures, it was almost impossible to count the number of eggs by driving her to one side in the bottle. By moving her

body backwards, she would attack once and again with open forceps. She would frequently catch my fine brush between her forceps. Invariably, while counting the eggs, I had to remove the female temporarily to another empty bottle and restore her to her eggs after my observations on the eggs were over which lasted about five minutes. When she is restored to her eggs, she either runs to the heap of eggs, or lie perplexed for a moment, so as to be thinking of her eggs as to where they were.

The eggs are highly susceptible to infection by fungus, for many are spoilt in spite of the mother's attention. In such cases the eggs either turn light brown, then dark brown or grey. The bad eggs are at once eaten by the mother. In spite of her solicitude for them the female will eat her eggs readily if conditions become too unfavourable or even if she is disturbed too much. When there is a dearth of sufficient humidity the eggs become dried and shrivel. Such eggs are readily eaten. On the other hand, mildewed eggs are reared by the mother for sometime. In one case the female was observed persistently with great devotion lying over her eggs for a pretty long time, although the eggs had become brown and although eggs laid contemporarily had hatched long before. She would even go back again to lie over them even when separated from the eggs. In this case the eggs had not lost their usual shape and size. This was an instance of remarkable adherence of the female to her eggs. In some instances, in spite of all precautions not to disturb the brooding female, she would devour her eggs, the reason for which could not be explained. In one instance, the female was brooding over 33 eggs from 19-5-1948 till 5-6-1948 and on 6-6-1948 not a trace of egg was left, all having been eaten overnight, although it was not starving, as dandelion blossoms were being supplied regularly. Besides, during the period of brooding, the female feeds very occasionally which indicates that probably the same is more true in case of females brooding in Nature, where they have to leave the nest in search of food.

Though the female earwig displays great parental care for eggs, she is not intelligent enough probably to our expectations. When a foreign matter similar in size and shape to her eggs is placed along with the egg mass she would mistake it to be one of her eggs and would carefully pile it with the eggs. The foreign matter is of course eventually rejected during the continued process of licking and cleaning. Even after discarding a foreign matter she may make the same blunder again. Once I put a small shell of a mollusc almost the size of an egg in an egg mass. The female during her shifting of eggs collected it with her eggs and eventually discarded it. I placed the shell again in the heap of eggs and it was rejected after being caressed for along with her eggs for sometime.

The male does not show any parental care and would devour the egg when he gets an opportunity.

#### MATERNAL CARE OF THE YOUNG

The opinion has been more than once expressed that the female ceases to care for her progeny after their hatching. Camerano's (1880) individual did not evince any interest in her young after they had hatched. The same view appears in Sedgwick's (1909) text book apparently based on works on the subject upto the time. Brindley (1914) termed the assertion that the female guards her young "to have no foundation". However, the earliest worker on the subject de Geer (1773) observed that the female earwig care for her young after birth. Fulton (1924, 1924a) believed that the nymphs normally remain in the nest until sometime after the first moult and by the third instar atleast, all family connections are broken. He recorded how the females guarded their young from the attacks of ground beetle larvae placed in the cages. Worthington

(1926) in England recorded that in no instance did a female desert her young until a considerable time after hatching. Crumb *et al* (1941) discovered that the young nymphs are attended by the mother earwig and remain in or near the original cell in the soil until they have spent a few days in the second nymphal instar, whereupon the parent relinquishes her responsibility for them. Guppy (1947) in British Columbia observed that the mother earwig remains in the cell with the nymphs for most of their first stadium. This review of literature would show that no two workers are in agreement on how long the mother earwig cares for her young.

In my observations I found that the mother earwig attended her young till after their second moult. In no case, the family tie is broken until the nymphs reach the adult stage. After the nymphs reach the second instar, mother's care for her young gradually decreases. But even after the nymphs are fairly grown up, at times of danger protection is sought by the young which the mother readily gives. This is evident from the fact that even when the nymphs are in the fourth instar, the mother would run to her young and push them under her at the slightest sense of danger. The nymphs also seem to sense the time of danger and run to the mother to huddle quietly under her.

Observations in the field also lent support to the above conclusions. On June 16, 1948 a female with 52 young nymphs were collected in the field at Edinburgh. Of these, atleast 16 were in the fourth instar and 17 were in the third instar.

I failed to notice any parental care of the male earwig. Chapman (1917) in England stated that they assisted in the care of the young earwigs. I have not come across any record substantiating Chapman's findings and believe this to be an erroneous statement.

Guppy (1947) stated that if the nymphs be deprived of their mother directly after hatching, they can fend for themselves, atleast if food is readily available. In my laboratory observations, although nymphs separated from the mother could be reared upto the adult stage, mortality was found to be high in them.

When a female lays a second batch of eggs (Behura, 1956), she ceases to show much interest for her young even before they have completed their second instar. In one particular instance, a female laid her second brood just a few days' after her young shed their skins for a second time. The nymphs however, at the slightest sense of danger would run to their mother and the mother readily gave them shelter under her abdomen.

#### SUMMARY

1. Although it is instinctive of the female earwig to build a nest, the male may occasionally carve out one.
2. At the time of oviposition, the male is expelled out of the nest. In the laboratory, there is some indication that in some cases, the female tolerated the presence of the male sometime after oviposition.
3. The female guards her eggs against intruders, lies over them, shifts them from one place to the other so as to keep them moist and yet free from fungus attack. Unless in a highly developed condition and about to hatch in two to three day's time, eggs in no case hatched in the absence of the female.

Mildewed eggs are eaten by the female earwig. If she is too much disturbed while brooding over the eggs, she eats away the eggs.

4. The mother earwig takes care of her young until late after their second moult although the family tie is not broken till late fourth instar. The nymphs take shelter under the mother at the slightest sense of danger.

When a female lays a second batch of eggs, she ceases to show much interest in her young even before they have completed the second instar.

5. The male shows no parental care and eats away the eggs whenever it gets an opportunity.

#### ACKNOWLEDGMENTS

The work was carried out at the Department of Zoology, University of Edinburgh, during 1948 and 1949, and it is with gratitude I acknowledge my indebtedness to Prof. James Ritchie for facilities I have enjoyed, and to Dr. B. M. Jones for his invaluable guidance. Thanks are due to the Government of Orissa for an overseas scholarship which enabled me to undertake this study.

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# STUDIES ON A DISEASE OF BAJRA (*Pennisetum typhoideum*) CAUSED BY *Curvularia penniseti*

By

G. P. AGARWAL

Department of Botany, Mahakoshal Mahavidyalaya, Jabalpur.

(Received on 26th June 1957)

## INTRODUCTION

*Pennisetum typhoideum* (called Bajra in Hindustani) is a tall erect grass belonging to the Natural Order Gramineae. It is used both as food and fodder and is commonly cultivated in North-West, Central and Southern India. Outside Asia the grain is largely cultivated in Africa where it is common article of food for the Arabs and the natives. It is generally sown in June or July and the crop is harvested in September or October.

Mitra (1921) first reported *Acrothecium penniseti* on Bajra from India. He observed that a parasitic species of *Acrothecium* was very common on Bajra at Pusa where it caused severe damage every year. He also observed that it was quite common at Kanpur, Hansi, Amritsar, Gurdaspur, Lahore and Lyallpur. He found it on diseased ears occasionally sent to Pusa from other places. Bertus (1948) reported that in Ceylon, the earhead disease of *Pennisetum typhoides* (*Syn-Pennisetum typhoideum*) was caused by *Acrothecium penniseti* in 1946. Boedjin (1933) separated *Curvularia* from *Acrothecium* and attributed certain distinctive characters to it, viz., conidia are more or less curved, third cell from the base of the conidium is disproportionately large and the end cell tends to remain more or less hyaline. Many species of *Acrothecium* were transferred to the newly erected genus *Curvularia*. *Acrothecium lunatum* Wakker was made the type species of the genus *Curvularia* as *C. lunata*. *A. penniseti* Mitra was designated as *C. penniseti*.

Mitra (1921) established the pathogenicity of *C. penniseti* (*Syn A. penniseti*) on leaves, leaf-sheaths and on the ears of *Pennisetum typhoideum*. He observed that infection could take place either through a stoma or by directly piercing an epidermal cell. He reported that the mycelium was both intra and inter cellular and was found in all parts of the infected leaf. During July and August 1952 the author examined certain Bajra fields at Allahabad and found a number of plants affected by the disease. *Curvularia penniseti* was invariably isolated from infected leaves and grains. Isolations from infected grains collected from a number of local stores also gave *C. penniseti*. Investigations of Mitra (l.c.) were limited to the vegetative parts of the plant. He neither studied the incidence of the disease on grains nor its effects on the germination of the seeds. Methods for the control of the disease were not tried. It was, therefore, decided to study the above aspects of the problem.

## MATERIAL AND METHOD

Affected grains, spotted and diseased leaves as well as leafsheaths of *Pennisetum typhoideum* were brought from local fields and isolations were made from them. In a majority of cases *Curvularia penniseti* was obtained. Single spore cultures of the above organism were prepared by the usual method.

For pathological studies Bajra seeds of different varieties were obtained from Dr. T. R. Mehta, Economic Botanist to the Government of Uttar Pradesh.

In order to test the pathogenicity of the fungus it was grown on sterilized seeds which were soaked in water for different periods. 0.1% mercuric chloride was used for sterilization and the grains were thoroughly washed after its use. In the earlier stages three different methods were tried but the method which gave most successful infection was employed in subsequent studies.

1. A suspension of the spores was prepared and sprayed on soaked seeds.
2. The soaked seeds were dipped in the spore suspension.
3. The soaked seeds were mixed with the mycelium and spores of the fungus.

For all soil inoculations a culture of the fungus was prepared on sand corn meal medium. 190 gms. of clean sand and 10 gms. of corn meal were taken in 500 c.c. Erlenmeyer's flasks. They were thoroughly mixed after adding water which was just sufficient to moisten the mixture. After autoclaving it was inoculated with the fungus which was allowed to grow for 15 days.

## OBSERVATIONS

*Symptoms of the disease*—The disease appeared in leaves, leafsheath, ears and on grains in storage. The symptoms of the disease on the vegetative parts as well as on the ears were similar to those described by Mitra (l.c.). The infection of the grain could start from any point and ultimately the organism covered the whole seed surface. The initial lesions on the seeds, appeared as dark brown small spots or patches without any definite shape but in later stages it surrounded the whole seed which became shrunken and black in colour.

## ARTIFICIAL INOCULATIONS

In order to test the pathogenicity of the fungus healthy grains were obtained from a local storage. They were soaked in the sterilized water for the following periods before inoculation :

5, 10, 15, 30, 45 and 60 minutes.

It was observed that in all cases where seeds were soaked for more than 5 minutes and were then kept for storage they started to germinate on the third day. Longer soaking was, therefore, unsuitable for testing the pathogenicity and the effect of the fungus on the germination of the grains. In all subsequent storage and germination experiments the seeds were soaked in water for 5 minutes only and were then



inoculated by the three methods described before. Controls were maintained in each case where the seeds were soaked in sterilized water but were not inoculated. The mycelium developed on all the inoculated series. There was no significant difference between the first two methods of inoculation which caused much less infection but it was more prominent when the seeds were mixed with the mycelium and spores of the fungus. In such cases infection appeared within 4 to 6 days after inoculation. Though the intensity of infection was greater on seeds soaked for 30 or 60 minutes, but such seeds could not be used for subsequent work because as mentioned before, they had started germinating and it was not possible to store infected grains or to test their germination after the infection.

In order to study the varietal differences, different varieties of seeds were inoculated and the intensity of infection at 24-25° C. was compared after 10 days. The results are recorded in Table No. 1.

**TABLE NO. 1**

*Showing infection of the seeds of different varieties*

Variety	Appearance of infection in days	Intensity of infection
Local	5	Severe
4105 Etawah	6	Poor
16/8 Partapgarh	5	Severe
4103/1 Hathras	6	Poor
EC 2168-C	6	Mild
C.F. Baroda	6	Poor
26 Mirzapur	5	Mild
Changoli Farm	5	Mild
Agra mixed	5	Severe
4 Bhingaon Poona	6	Mild
12 Peshawar	6	Poor

It is clear from the above table that there was distinct varietal difference as some varieties were more severely infected than the others but none of them were immune. The least infection was on 4103-1 Hathras, C.F. Baroda and 13 Peshawar.

**Effect of different temperatures.** In order to study the effect of different temperatures on the incidence of the disease the grains of the local variety were inoculated

and kept at 10, 15, 20, 25, 30 and 35°C. It was observed that the grains were not infected at 10°C. At 15°C infection started after 8 days and progress of the disease was very slow. At 20 and 25°C the infection started after 5—6 days and was severe within 10—12 days. The disease spread most rapidly at 30°C. At that temperature the infection started after 4 days and was severe within 8 days. The initial infection at 30°C was similar to that at 30°C but the progress of the disease was relatively slow as it became seriously infected after 10 days.

**Effects of the fungus on germination.** In order to study the effect of the fungus on the germination of the grains healthy, slightly as well as heavily infected seeds were grown in petri dishes. Five seeds were kept in each petri dish and four replicates were taken. The results are summarized in Table No. 2.

**TABLE NO. 2.**

*Showing germination of healthy, very slightly and half infected seeds of different varieties grown in petri dishes.*

Variety	Percentage germination		
	Healthy seeds	Very slightly infected seeds	Half infected seeds
Local	100	100	45
4105 Etawah	100	100	60
16-8 Partapgarh	100	95	50
4103-1 Hathras	100	100	55
E C 2168-C	95	100	55
C.F. Baroda	100	95	60
26 Mirzapur	100	90	50
Changoli Farm	95	100	55
Agra mixed	100	95	45
4 Bhingaon Poona	100	95	50
12 Peshawar	100	100	60

The above table shows that there was no difference in the germination of healthy and very slightly infected seeds but the germination of half infected grains was distinctly poor.

The seeds of different varieties were also sown in healthy and infected soil. In order to prepare artificially infected soil, some sterilized soil was inoculated by sand corn meal cultures of *C. penniseti*. Infected as well as healthy seeds were sown in separate pots containing sterilized and contaminated soil. Only slightly infected seeds of different varieties with more or less similar amount of infection were used in the present investigation. Thirty seeds of each type were sown in a pot and three replicates were taken. The results are summarized in Table No. 3.

TABLE NO. 3.

*Showing germination of healthy and slightly infected seeds of different varieties on sterilized and contaminated soil*

Variety	Percentage germination of seeds			
	Healthy seeds in sterilized soil	Healthy seeds in infected soil	Infected seeds in sterilized soil	Infected seeds in infected soil
Local	96.7	100.0	96.7	93.3
4105 Etawah	96.7	96.7	90.0	100.0
16/8 Partapgarh	100.0	100.0	93.3	96.7
4103/1 Hathras	100.0	100.0	100.0	100.0
E C-2168-C	100.0	96.7	100.0	93.3
C.F. Baroda	93.3	100.0	90.0	90.0
26 Mirzapur	100.0	100.0	96.7	93.3
Changoli Farm	96.7	93.3	93.3	96.7
Agra Mixed	100.0	96.7	100.0	90.0
4 Bhingaon Poona	100.0	100.0	93.3	100.0
12 Peshawar	96.7	100.0	90.0	96.7

Table No. 3 shows that there was almost no difference in the germination of seeds in sterilized or infected soil. Thus the presence of the fungus in the soil did not retard the germination of the grains. There was no appreciable difference in the germination of healthy or slightly infected seeds and in this respect the results were similar to those obtained in the petri dish experiment. The seedlings from slightly infected seeds were equally healthy and were exactly like those from the normal healthy seeds. Thus it is fully confirmed that the slight infection of the seeds had no adverse effects on its germination.

In the above experiment only slightly infected seeds were taken but the experiment conducted in petri dishes indicated that the germination of seeds could depend upon the severity of infection. The previous experiment was, therefore, repeated with seeds showing different intensities of infection. The previous experiment also indicated that there was no difference in the germination in healthy or infected soil. It was, therefore, decided to use sterilized soil only.

he following two types of seeds were tested :

1. Nearly half the grain surface infected.
2. Completely infected grains.

The results are recorded in Table No. 4.

**TABLE NO. 4.**

*Showing the germination of healthy seeds as well as of the seeds showing different degrees of infection.*

Variety	Percentage germination		
	Healthy seeds	Half infected	Completely infected
Local	98	62	24
4105 Etawah	100	60	26
16/8 Partapgarh	96	66	28
4103/1 Hathras	94	62	24
E. C. 2168-C	100	70	24
C.F. Baroda	90	62	20
26 Mirzapur	94	68	24
Changoli Farm	98	70	28
Agra Mixed	100	64	20
4 Bhingaon Poona	96	64	20
12 Peshawar	100	68	24

It is evident from the above table that the germination of half infected seeds was reduced to 60-70 per cent. There was considerable reduction in the percentage germination of completely infected seeds which ranged from 20 to 28% in different varieties. It was also noticed that the loss in the germination percentage depended upon the severity of infection and the effect due to varietal differences of the seeds was not significant. The seedlings developed from the healthy and half infected seeds were similar while those from the completely infected seeds were definitely weaker and smaller.

*Infection in storage*—The effect of the infected grains on the healthy ones in storage was also studied. For this purpose healthy grains of the local variety were surface sterilized by the usual method and they were mixed with the infected grains after which they were stored at different relative humidities which were adjusted by the method suggested by Clayton (1942). The following humidities were used 100, 95, 88, 65, 32 and 0% and the spread of the disease was carefully noted. It was found that at the highest relative humidity i.e. at 100% the disease appeared earliest and was noticed after 7-8 days. At 95% the infection appeared after 9 days. The infection was delayed at 88% where it appeared after 10-12 days. There was no infection at 0, 32, and 65%. Thus it is seen that the diseased grains could cause infection in storage at high relative humidities only.

#### CONTROL METHODS

An attempt was made to control the disease by treating the seeds with a number of common fungicides. The following ones were used :—

Copper Sulphate, Perenox, Formalin dust, Agrosan G.N., Spergon, Mercuric Chloride, Ceresan, Zerlate, and Diathane Z-78.

Surface sterilized, healthy grains of the local variety were taken in sterilized conical flasks and the above fungicides (0.25% seed weight) were mixed with them. The percentage of fungicides taken was according to the standard recommendations of the manufacturers as reported by Jacks (1952). One set was kept as control in which no fungicide was added. After two days the seeds were inoculated and the observations were continued for a fortnight.

It was noticed that in the control set infection started after 5 days and was severe within 10 days. Most of the fungicides showed improvement in the intensity of infection on the seeds. Zerlate and Diathane Z-78 were not effective and showed no improvement over the control. Perenox, Agrosan G.N., Spergon and Ceresan considerably decreased the infection which was less in intensity and was delayed as it appeared after 7-8 days, but it could not be completely checked. Seeds treated with copper sulphate, formalin dust and mercuric chloride were free from infection and they could satisfactorily control the disease.

The effect of applying the fungicides after inoculating the seeds was also studied, The seeds were treated in the following manner :—

1. Fungicides applied immediately after inoculation.
2. Fungicides applied 2 days after inoculation.
3. Fungicides applied seven days after inoculation.

It was found that as in the previous case Zerlate and Diathane Z-78 were unable to control the spread of the disease in any case. Perenox, Agrosan G.N., Spergon and Ceresan decreased the intensity of infection in the first two series but the infection appeared if they were applied seven days after inoculation. There was no infection in seeds treated with copper sulphate, formalin dust and mercuric chloride provided the fungicides were applied within two days of inoculation. In the third set the infection had already spread considerably before the seeds were treated with the fungicides. In this case even copper sulphate, formalin dust, and mercuric chloride could

not check the disease but its progress was considerably retarded and the intensity of infection was less than in untreated control. In every other case of this series the infection was severe within 10-11 days.

The effect of the fungicides on the germination of healthy and half infected seeds was also studied. Healthy as well as nearly half infected seeds were treated with the fungicides as described earlier and after two days they were sown in pots filled with sterilized soil. One set of the infected seeds without treating with any fungicides was kept as control.

Their percentage germination was studied and it was noticed that the fungicides excepting copper sulphate had no adverse effect on the germination of the healthy seeds. Copper sulphate greatly depressed the germination of healthy as well as infected seeds, and hence it was considered unsuitable. It was observed that none of the fungicides could check the trouble. Slight improvement was noticed when formalin dust, Agrosan G.N. and mercuric chloride were used. It appears that even half infected seeds were sufficiently damaged and hence their percentage of germination could not be improved.

It is concluded that the seeds should be stored under dry conditions and the relative humidity should not be allowed to increase beyond 65%. In view of the decreased germination of the infected grains it is desirable to use suitable fungicides for the seeds reserved for sowing purposes. Mercuric Chloride and formalin dust prevent the spread of the disease but proper care should be taken to prevent the use of treated grains for food purposes. These should be used only for the seeds which may be stored for sowing.

#### SUMMARY

*Curvularia penniseti* attacks leaves, leafsheath, and ears of Bajra (*Pennisetum typhoides*). Its pathogenicity on the grains was established. Different varieties of Bajra were tested but none was found to be immune. Some varietal differences were, however, noticed. The effect of different temperatures on the spread of the disease on the grains was studied. Infection could not take place at 10° C. The optimum temperature for the spread of the disease was found to be 30° C. Slight infection of the seeds did not retard germination but it was considerably reduced in severely infected seeds. The presence of the fungus in the soil did not retard the germination of the grains. The diseased grains could cause infection in storage at high relative humidities only. Treatment of the seeds with Perenox, Agrosan G.N., Spergon and Ceresan considerably decreased the spread of the disease though it was not completely checked. The seeds treated with copper sulphate, formalin dust and mercuric chloride were almost free from infection. Copper sulphate, however, retarded the germination percentage of healthy seeds. Treatment of seeds with fungicides after they were severely infected could not improve their percentage of germination.

#### ACKNOWLEDGMENTS

I express my deep gratitude to Dr. R. N. Tandon for his guidance and for critically reading the manuscript of this paper. My thanks are also due to Professor S. Ranjan for providing the necessary laboratory facilities during the course of this

work which was carried on in the Botanical laboratories, University of Allahabad. Thanks are also due to Dr. T. R. Mehta for providing the various varieties of *Pennisetum typhoideum*.

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# CULTURAL STUDIES OF *PESTALOTIA MANGIFERAE* (BUTL) WITH SPECIAL REFERENCE TO ITS SPORULATION I. THE INFLUENCE OF NUTRIENTS

By

R. N. TANDON and K. S. BILGRAMI

*Department of Botany, University of Allahabad.*

(Received on 12th July, 1957)

Sporulation of fungi is greatly dependent on nutrition. Sources of carbon, nitrogen, sulphur, vitamins and initial pH of the medium are some of the important factors which determine the amount of sporulation of many organisms. During the last decade various workers including Barnett and Lilly (1947), Buston and Basu (1948), Hawker (1948), Mathur et al (1950), Tandon and Bilgrami (1954), Grewal (1954) and Bilgrami (1956) have studied the role of nutrients on the sporulation of various fungi. Preliminary studies had shown that *Pestalotia mangiferae* could easily produce abundant spores on a number of synthetic media and even slightest modification in the constituents of the culture medium greatly influenced the degree of sporulation. It was, therefore, decided to select this fungus as a test organism for more detailed studies.

## MATERIAL AND METHOD

The organism was isolated from the infected leaves of *Mangifera indica*. Asthana and Hawker's medium A<sup>1</sup> which supported maximum sporulation was used as the basal medium. Garrett's (1936) agar disc method was used for inoculations, except in the experiment dealing with vitamin requirements, where the inoculations were made by adding 0.1 c.c. spore suspension (containing about 50 spores). Pyrex glass wares and purest available chemicals were used. Studies on pH indicated that the growth and sporulation was best at pH 6.0. It was, therefore, decided to adjust the pH at 6.0 in all cases except in the experiment dealing with the influence of pH. The media were dispensed into 150 ml Erlenmeyer flasks in 25 ml quantities. Autoclaving was carried out at 15 lbs pressure for 15 mts, except where otherwise indicated. Cultures were incubated for 15 days at 25°C, after which the entire fungal mat was harvested by filtering over previously weighed and dried Whatman's filter paper No. 42. The method of calculating the dry weight was similar to that of Tandon and Bilgrami (1954).

The pH of media was adjusted with the help of hydrochloric acid potassium hydroxide and for the study of its effect it was adjusted to different values from pH 2.0 to pH 9.0.

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1. dextrose 5.0 gms, KNO<sub>3</sub> 3.5 gms, KH<sub>2</sub>PO<sub>4</sub> 1.75 gms, MgSO<sub>4</sub> 7H<sub>2</sub>O, 0.75 gm, distilled water 1 litre (2% agar was added to solidify the media, whenever so desired).



In order to determine the effect of various combinations of different carbon and nitrogen sources on growth and rate of sporulation, the quantities of glucose and potassium nitrate of Asthana and Hawker's medium A were replaced by other carbon and nitrogen sources respectively. The amount of these substances was so adjusted as to supply the same quantity of carbon or nitrogen which was present in the basal medium. Various combinations of 7 carbon and 7 nitrogen sources were used (i.e. 49 in all). The effect of different sources of sulphur was also studied and for this purpose various sulphur compounds were substituted singly in place of magnesium sulphate of the basal medium. The amount of sulphur which was supplied was similar to that of 0.75 gm of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .

Role of various concentrations of five different vitamins was also studied. The method employed was similar to that of Tandon and Bilgrami (1957).

The degree of sporulation has been indicated on the basis of number of spores present under the low power field of the microscope and they have been grouped into following categories.

<i>Number of Spores</i>	<i>Sporulation.</i>
Nil	Absent
1—10	Poor
11—20	Fair
21—30	Good
Above 30	Excellent

For grading the dry weight into good, moderate and poor, the general mean of the experiment  $\pm$  critical difference at 1% level has been reported as moderate. The dry weights higher than moderate have been designated as good and lower ones as poor.

## EXPERIMENTAL

(i) **Influence of different pH concentrations** :—The hydrogen ion concentration of the basal medium was varied from pH 2.0 to 9.0 and 12 different pH values were used. It was found that pH 5.5-6.5 was best for the sporulation as well as growth. Highly acidic or highly alkaline media (viz pH 3.0, pH 9.0 respectively) completely suppressed sporulation though they permitted feeble growth.

(ii) **Influence of different combinations of various sources of carbon and nitrogen** :—In this case the sporulation was noted after every 5th day but the dry weight was recorded after the completion of the incubation period i.e. 15 days. The results are, therefore, recorded separately in tables 1 and 2 respectively. The purpose of noting the sporulation after every 5th day was to determine the best carbon-nitrogen combination for maximum sporulation in the shortest time. On the basis of previous results of Bilgrami (l.c.) both good and poor sources of carbon and nitrogen were included for this study. The following compounds were used.

*Carbon sources*—Sucrose, maltose, lactose, glucose, rhamnose, glycerol and malic acid.

*Nitrogen sources* :—dl valine, glutamic acid, potassium nitrate, arginine, asparagin, glycine and ammonium nitrate.

Steam sterilization was carried out on two different days for media containing the disaccharides (viz. sucrose, maltose or lactose).

TABLE NO. 1

*Showing rate of sporulation of Pestalotia mangiferae on combinations of various carbon and nitrogen sources.*

Carbon compounds	Incubation period DAYS	NITROGEN SOURCES						
		dl-valine	Glutamic acid	KNC <sub>3</sub>	Arginine	Asparagin	Glycine	Amm. nitrate
Sucrose	5	Fair	Good	Good	Fair	Poor	Absent	Absent
	10	Good	Excellent	Excellent	Good	Fair	Absent	"
	15	Good	Excellent	Excellent	Good	Fair	Poor	"
Maltose	5	Fair	Fair	Fair	Fair	Poor	Absent	"
	10	Good	Good	Good	Good	Poor	Absent	"
	15	Good	Excellent	Excellent	Good	Poor	Poor	"
Lactose	5	Absent	Absent	Absent	Absent	Absent	Absent	"
	10	Absent	Absent	Absent	Absent	Absent	Absent	"
	15	Absent	Absent	Absent	Absent	Absent	Absent	"
Glucose	5	Fair	Good	Good	Fair	Absent	Absent	"
	10	Good	Excellent	Excellent	Good	Poor	Absent	"
	15	Good	Excellent	Excellent	Good	Poor	Absent	"
Rhamnose	5	Absent	Poor	Absent	Poor	Absent	Absent	"
	10	Poor	Fair	Poor	Poor	Absent	Absent	"
	15	Poor	Fair	Poor	Poor	Poor	Absent	"
Glycerol	5	Poor	Poor	Poor	Poor	Absent	Absent	"
	10	Fair	Fair	Fair	Fair	Poor	Absent	"
	15	Fair	Good	Fair	Fair	Fair	Absent	"
Malic acid	5	No growth	.....	.....	.....	.....	.....	.....
	10	No growth	.....	.....	.....	.....	.....	.....
	15	No growth	.....	.....	.....	.....	.....	.....

A reference to Table No. 1 shows that both carbon and nitrogen sources were jointly responsible for determining the rate of sporulation of *P. mangiferae*. It is evident from the above table that any combination of nitrogen with lactose was incapable of supporting sporulation of this organism. Amongst the nitrogen sources combination of ammonium nitrate with any carbon source completely inhibited the fruiting (vide plate I, fig. 1). A combination of glutamic acid or potassium nitrate with maltose, sucrose or glucose were excellent sources for development of its spores, (vide plate I, fig. 2). Combinations of dl-valine or arginine with above mentioned carbon compounds served as good sources (vide plate II, fig. 3), but their combinations with rhamnose and glycerol were poor and fair sources respectively. Asparagin in combination with sucrose or glycerol supported fair sporulation, but it remained a poor source with other combinations of carbon. Glycine induced poor sporulation when mixed with sucrose or maltose, though it totally suppressed the spore production with other carbon sources. The above results further indicate that the rate of sporulation also varied with the carbon nitrogen combination. The sporulation could change after every 5th day e.g. (maltose-glutamic acid) or could remain stationary from 6th-16th day e.g. (maltose-asparagin). In certain cases the sporulation increased upto 10th day and then remained stationary e.g. (maltose-dl valine), while in others the spores were not produced till 10th day and appeared after that period e.g. (maltose-glycine).

TABLE NO. 2

*Showing dry weight in mg of Pestalotia mangiferae on combinations of various carbon and nitrogen sources.*

Carbon sources	NITROGEN SOURCES						
	dl valine	glutamic acid	Potassium nitrate	arginine	asparagin	glycine	amm. nitrate
Sucrose	125.0	119.0	67.0	47.0	74.0	39.0	119.0
Maltose	122.0	141.0	74.0	43.0	63.0	36.0	109.0
Lactose	57.0	55.0	39.0	34.0	43.0	24.0	51.0
Glucose	121.0	106.0	62.0	55.0	61.0	40.0	118.0
Rhamnose	61.0	50.0	32.0	38.0	39.0	20.0	44.0
Glycerol	49.0	50.0	38.0	30.0	46.0	19.0	53.0
Malic acid	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Average = 53.3

Summary of dry weight results and conclusions at 1% level of P.

Treatment	..	highly significant
Replicates	..	non significant
S.E.	..	C.D. at 1% level
1.751	..	6.5

It is evident from the above table that these carbon, nitrogen variables not only effected the sporulation, but they markedly influenced the growth also. It was found that dl valine, glutamic acid, asparagin, potassium nitrate or ammonium nitrate in combination with maltose, sucrose or glucose served as satisfactory carbon-nitrogen combinations for this fungus. The growth on rhamnose, lactose or glycerol slightly improved in combination with dl-valine or glutamic acid. Malic acid failed to induce any growth even when it was combined with very good nitrogen sources. Best growth of *Pestalotia mangiferae* was attained when maltose-glutamic acid were used as carbon nitrogen sources.

A critical study of tables Nos. 1 and 2 established that there was no correlation between growth and sporulation. A particular combination of carbon and nitrogen may be good both for growth and sporulation or excellent for growth and poor for sporulation. In general the sporulation was not good when the growth was poor.

(iii) **Influence of different sources of sulphur** :— The effect of 10 different sources of sulphur was also studied and it was found that only magnesium sulphate was capable of supporting excellent sporulation. Potassium sulphate, sodium bisulphate, thiourea, cystein and sodium bisulphite supported poor sporulation only. Ammonium sulphate, sodium thiosulphate and potassium persulphate did not permit the development of spores.

Statistical analysis of the dry weight results showed that magnesium sulphate and cystein were best sulphur sources for the growth of *P. mangiferae*. This fungus was also capable of producing good mycelial growth on potassium sulphate, sodium bisulphate, sodium thiosulphate and ammonium sulphate. It is clear that even though the last two support good growth they are quite unsuitable for sporulation. Thiourea and potassium persulphate were found to be poor sources of sulphur, while it was incapable of growing on zinc sulphate and sodium bisulphite.

(iv) **Influence of vitamins** :— Six different vitamins viz. B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, C, H and nicotinic acid were added at the rate of 10, 50, 100, 200 and 500  $\mu$ gm per litre in the vitamin free basal medium and their reaction was studied.

Table three shows that *Pestalotia mangiferae* was incapable of producing spores on vitamin free media or when nicotinic acid was added to the medium, while vitamin C could induce only poor sporulation. Fair sporulation of this organism was recorded on thiamin. Sporulation on riboflavin and biotin increased with their concentration as their higher doses were good for sporulation. Only pyridoxine supported excellent sporulation, when the concentration of this vitamin was more than 100  $\mu$ gm per litre. Dry weight results showed that maximum growth of this organism was attained at highest concentration of riboflavin (vitamin B<sub>2</sub>) which was followed by thiamin and biotin. Growth at lower concentrations of pyridoxine was better than on similar concentrations of biotin, but higher concentrations of this vitamin (pyridoxine) viz. 200 and 500  $\mu$ gm per litre decreased the growth of this fungus. Very



Fig. 1

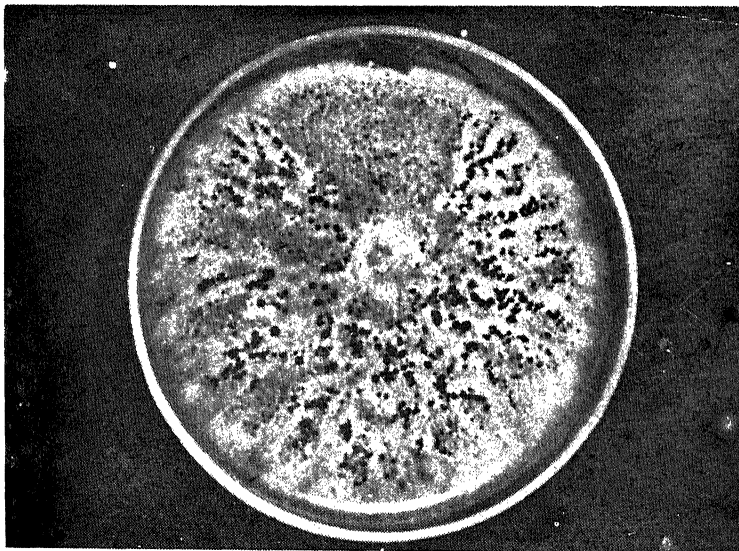
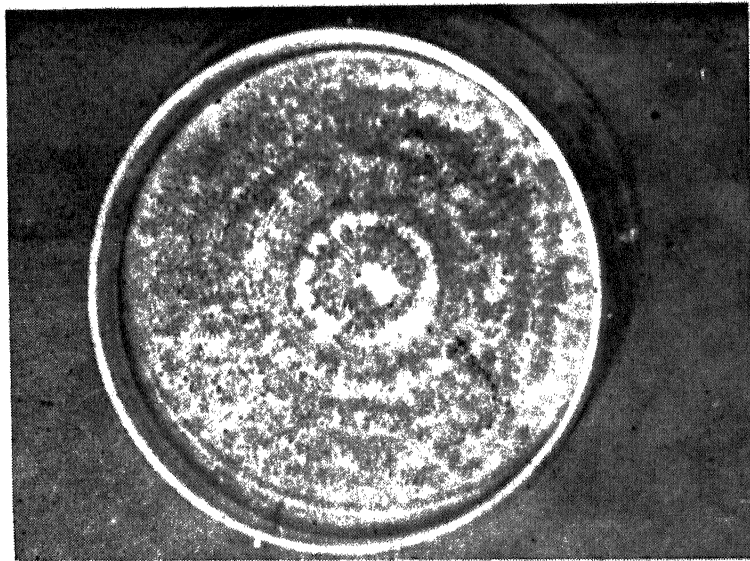


Fig. 2

Plate I



**Fig. 3**

**Plate II**

TABLE NO. 3

Showing average growth in mgs. and sporulation of *Pestalotia mangiferae* at different concentrations of six vitamins. The nature of sporulation is indicated in brackets below the dry weights.

vitamins added	Amount of vitamins in $\mu$ gm. per litre					Average
	10	50	100	200	500	
Thiamin (Vitamin B <sub>1</sub> )	49.0 (Poor)	56.0 (Fair .....	68.0	79.0	89.0	68.0
Riboflavin (B <sub>2</sub> )	57.0 (Fair .....	66.0	74.0	85.0 (Good .....	96.0	75.6
Pyridoxine (B <sub>6</sub> )	51.0 (Good .....	55.0	66.0 (Excellent.....)	64.0	56.0	58.4
Biotin (H)	45.0 (Fair .....	52.0	61.0 (Good .....	71.0	80.0	61.8
Ascorbic Acid (C)	46.0 (Poor.....)	49.0	44.0	41.0	33.0	42.6
Nicotinic acid	45.0 (Absent.....)	47.0	42.0	37.0	30.0	40.2
Control	42.0 (Absent.....)	43.0	41.0	40.0	43.0	42.8
Average	47.8	52.5	56.5	59.5	61.0	41.8

Treatment	S.E.	C.D. at% level
Vitamins	0.88	3.3
Concentrations	0.624	2.336
	0.765	2.862

**Vitamin treatments**

Vitamin  
Dry wt. in mgs.

B<sub>2</sub> > B<sub>1</sub> > H > B<sub>6</sub> C Control  
75.6 > 68.2 > 61.8 > 58.4 42.6 41.8

Nicotinic acid  
40.2

**Concentration treatments**

Vitamin Concentration  
Dry wt. in Mgs.

500 > 200 > 100 > 50 > 10  
61.0 > 59.5 > 56.5 > 52.5 > 47.8

slight increase in growth was also recorded on lower concentrations of vitamin C and nicotinic acid. Higher concentrations of these two vitamins were toxic for the growth of the present organism.

## DISCUSSION

Spores are more resistant to the seasonal variations than other parts of the fungal body. Under natural conditions it was found that numerous pseudopycnidia of *Pestalotia mangiferae* were developed on the upper surface of the diseased mango leaves. Spores of the pathogenic fungi may be responsible for the carry over of the disease from year to year. It was, therefore, essential to determine the factors which facilitate or inhibit the production of spores.

The results established that the best pH range for the sporulation of *P. mangiferae* was between 5.5-6.5. The pH of mango leaf extract was 5.7 and this organism could, therefore, easily sporulate on the host tissue because the pH of the mango leaves was quite favourable.

Analysis of the host contents revealed the presence of nitrates, amides, several amino acids and various sugars in the leaves of *Mangifera indica*. This indicated that under natural conditions different sources of carbon and nitrogen were available to the fungus in various combinations. Best carbon-nitrogen combinations for the sporulation of the present organism were, therefore, determined. Timnick et al (1951) reported that a combination of maltose-ammonium tartarate was best for the sporulation of *Melanconium fuligenium*. They further observed that lactose and starch were poor carbon sources, irrespective of the nitrogen source. Similarly urea and potassium were poor nitrogen sources and their combination with good sources of carbon could not improve the sporulation. The present results also agreed with those of Timnick et al (1951) and it was found that combination of poor nitrogen source with good carbon source or vice versa improved the growth or the sporulation to some extent but in all such cases the effect of poor source retained its influence. It is, therefore, clear that best results for growth and sporulation can only be obtained when best sources of carbon and nitrogen are combined together and none of them can singly make up for the poor quality of the other.

There is very little work on the effect of different sources of sulphur on the sporulation of fungi. The present results clearly established that with the exception of magnesium sulphate, the other sources of sulphur either completely inhibited the spore production or induced only poor sporulation.

The importance of vitamins in the sporulation of *Pestalotia mangiferae* was exhibited by the fact that spores were not formed in vitamin free media. Barnett and Lilly (1947) reported that the perithecial production of *Sordaria fimicola* could be controlled by the amount of thiamin. Tandon and Bilgrami (1957) reported that even lowest concentration of thiamin (viz. 10 µgm per litre) could cause excellent sporulation of *Phyllosticta artocarpina*. The present fungus differed from those organisms as even highest concentration of thiamin (viz. 500 µgm per litre) induced only fair sporulation. Higher concentrations of biotin (vitamin H) and riboflavin (vitamin B<sub>2</sub>) supported good sporulation of *P. mangiferae*. Grewal (1954) and Agrwala (1955) obtained similar results with this vitamin for *Gloeosporium musarum* and *G. psidii* respectively. Excellent sporulation of the present fungus was obtained only at the



higher concentrations of pyridoxine (vitamin B<sub>6</sub>). Nicotinic acid was incapable of inducing any sporulation, while ascorbic acid supported poor development of spores. *Gloeosporium papayae* investigated by Grewal (1954) gave similar results with ascorbic acid.

Mathur et al (1950) , Timnick et al (1951), Hawker (1948) and Agarwal (1955) mentioned that several nutritional factors were jointly responsible for controlling the sporulation of fungi. These observations are fully confirmed by the present investigation.

## SUMMARY

1. The influence of various nutrients on the sporulation of *Pestalotia mangiferae* (Butl) was studied. It was found that a pH between 5.5 to 6.5 was best for the development of spores.

2. Sources of carbon and nitrogen markedly effected the sporulation. A combination of potassium nitrate or glutamic acid with maltose, sucrose or glucose proved excellent for sporulation. Combinations of lactose with any source of nitrogen or of ammonium nitrate with any source of carbon completely inhibited the sporulation.

3. Magnesium sulphate was found to be an excellent source of sulphur for sporulation.

4. The organism failed to produce spores in a vitamin free medium or when nicotinic acid was used as a source of vitamin. Pyridoxine induced excellent sporulation. Riboflavin and biotin were good sources while thiamin supported only fair sporulation which was poor in presence of ascorbic acid.

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## Explanation of the Plates

- Plate I Fig. 1 No sporulation of *P. mangiferae* on maltose-ammonium nitrate agar.  
 Plate I Fig. 2 Excellent sporulation of *P. mangiferae* on sucrose-KNO<sub>3</sub> agar.  
 Plate II Fig. 3 Good sporulation of *P. mangiferae* on sucrose-arginine agar.

# MORPHOLOGY OF THE ALIMENTARY CANAL OF THE MOTH LEUCINODES ORBONALIS GUEN (LEPIDOPTERA, PYRAUSTIDAE)

By

B. P. SRIVASTAVA

*Department of Zoology, University of Allahabad.\**

*(Received on 24th December, 1956)*

## INTRODUCTION

The study of the Lepidopterous digestive system has interested several workers but most of them have confined their attention to the postembryonic development and the metamorphosis of the alimentary canal and there exist very few comprehensive accounts on the anatomy and the histology of the gut of the imago. Burgess (1880) was the first to give an accurate account of the anatomy of the digestive system in *Darius archippus* (Danaiidae). Bordas (1920) made extensive studies on the alimentary canal and its associated structures in several species of Papilionidae, Nymphalidae, Satyridae, Notodontidae, Saturnidae and Noctuidae. His contention of the process of secretion is still a controversial issue. Pyle (1940) studied the digestive system in *Callosamia promathea* (Saturnidae) which is a non-feeding moth and compared it with the feeding forms. Quite recently, however, Waterhouse (1953) reported the occurrence of the peritrophic membrane in several adult Lepidoptera which was so far regarded absent in the fluid feeding insects. Hence the present work has been undertaken with a view to clear some of the existing controversies and to add to the available literature a detailed account of the morphology of the alimentary canal of *L. orbonalis* as a representative of Pyraloidea which has so far escaped the attention of the past workers.

## MATERIAL AND TECHNIQUE

Since it is difficult to trap the moths in the field they were reared in the laboratory from the brinjals infected with the larvae. The moths were dissected in physiological salt solution and the various parts of the alimentary canal were fixed, dehydrated and sectioned in usual manner at 6 to 8 micra thickness. The sections were stained in in Delafield's haematoxylin and eosin.

## OBSERVATIONS

The alimentary canal of the imago (Fig. 1) can be divided into three regions, viz. the foregut or stomaeum, the midgut or mesenteron and the hindgut or proctodaeum. The gut of the imago is about double the length of the insect itself. The

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\*At present Agricultural Institute, Jobner, Jaipur, Rajasthan.

foregut is quite long and continues upto the first abdominal segment. The midgut is comparatively smaller and extends from the first to the fourth abdominal segment, lying a little obliquely. The hindgut is also very long and lies coiled in the posterior abdominal segments. It opens to the exterior through a narrow slit between the uncus and the ninth sternum in the male and between the two ovipositor lobes in the female.

### THE FOREGUT

The foregut can be conveniently divided into four different regions viz., the pharynx, the oesophagus, the crop and the oesophageal valve, which are clearly distinguishable from one another anatomically (Fig.1) and histologically. The ball-shaped pharynx opens anteriorly to the exterior through the proboscis. Posteriorly it continues

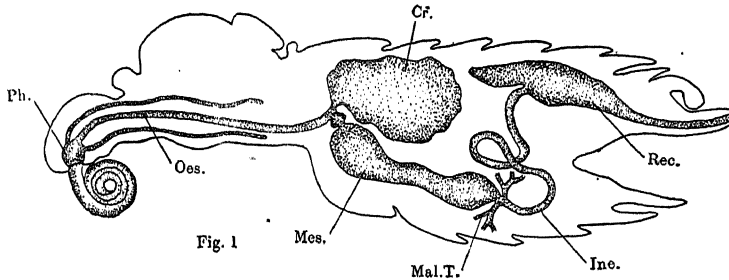


Fig. 1

Fig. 1. Gross anatomy of the alimentary canal of the imago.

Cr., crop; Ine., intestine; Mal. T., malpighian tubule; Mes., mesenteron; Oes., oesophagus; Ph., pharynx; Rec., rectum.

into a slender tubular oesophagus beyond the thoracic segments. The crop is attached to the posterior portion of the oesophagus and is in the form of a dilated sac-like structure which is connected to the latter by a short, narrow duct. The crop has been variably termed as the jabot, the sucking stomach, etc. At the junction of the foregut and the midgut is an oesophageal valve which is a homologue of the proventriculus of generalized insects.

Proceeding from the outer to the inner side, the wall of the foregut consists of the following layers :—

- (1) A thin sheet of connective tissue.
- (2) layers of muscle fibres,
- (3) a single layer of epithelial cells situated on a basement membrane, and
- (4) the chitinous intima.

The layer of longitudinal muscles is internal to that of the circular ones.

*The Pharynx.* It is the anterior most region of the alimentary canal and occupies the region of the head between the galea and the cerebral ganglionic mass. Its

wide lumen is provided with a thick chitinous lining which is secreted by a thin layer of flat epithelium (Fig.2). The cell walls are indistinct and deeply staining nuclei are quite large and prominent. The cytoplasm is granular and the thin structureless basement membrane is quite distinct. The layers of circular and longitudinal muscle fibres are very well developed.

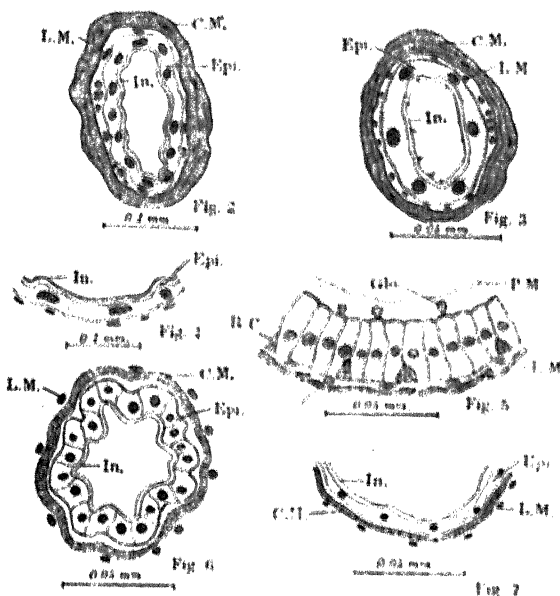


Fig. 2

Figs. 2—7; Fig. 2, T. S. of the pharynx ; Fig. 3, T. S. of the oesophagus ; Fig. 4, T. S. of a portion of the crop; Fig. 5, L. S. of a portion of midgut in its anterior region; Fig. 6, T. S. of the intestine; Fig. 7, T. S. of a portion of the rectum.

C. M., circular muscles; Epi., epithelium; Glo., globular extrusions; In., chitinous intima; L. M., longitudinal muscles; P. M., peritrophic membrane; R. C., regenerative cells.

*The Oesophagus.* The anterior region of the thin tubular oesophagus passes through the fused mass of the supraoesophageal and suboesophageal ganglia. When passing through the ganglionic mass, the oesophagus is lateroventrally compressed so as to appear conical in shape. In the thoracic cavity it runs side by side with the dorsal blood vessel. Anterior to the aortic arch both the dorsal blood vessel and the oesophagus are attached by a common membrane of connective tissue and the two run like that above the lowest layer of the longitudinal thoracic muscles. Here the dorsal blood vessel gets dilated to form the aortic arch and gets separated from the oesophagus which continues posteriorly resting above these muscles.

The lumen of the oesophagus (Fig.3) is very narrow and irregular and the chitinous intima, which is quite prominent, is provided with small spine like processes. The layer of flat epithelial cells very much resembles that of the pharynx. The basement membrane is very thin and is in close contact with the epithelium. Musculature is thick and the circular muscles are external to the longitudinal ones.

*The Crop.* It is commonly known as the sucking stomach in Lepidoptera and is attached to the posterior portion of the oesophagus through a small duct which

enters the oesophagus a little anterior to the oesophageal valve. In a newly emerged imago, it remains inflated with air and occupies the entire dorsal portion of the first three and a part of the fourth abdominal segment, possibly to assist the insect in breaking the pupal case when the imago is about to emerge. In an old imago it gets compressed and is thrown into several irregular transverse and longitudinal folds.

The wide lumen of the crop is lined with prominent chitinous intima and the layer of the flat epithelial cells is very thin (Fig.4). The cells are not provided with boundaries and fairly large deeply staining oval or round nuclei cause local bulgings of the epithelium. The basement membrane is thin with the muscle fibres widely separated.

*The Oesophageal Valve.* The oesophagus is telescoped into the midgut in such a manner as to form the oesophageal valve. It is a cylindrical fold of the foregut projecting into the lumen of the midgut and thus forming a double layered fold. The intima of the foregut is continuous over the oesophageal valve which joins the anterior of the midgut. The blood sinus, which has been reported to be present in many adult insects at the junction of the fore and the midgut, is absent in this moth. The posterior edge of the oesophageal valve joins the midgut vertically. In transverse sections through this region three layers of cells are visible. The outer one is that of the midgut epithelium while the inner two are those of the foregut.

### THE MIDGUT

The length and the width of the midgut are very variable. In the male it is supported by loose connective tissue and the fat body while in the female it is very much compressed because of the presence of large ovaries.

The columnar epithelium (Fig.5) which is provided with low striated border has a granular cytoplasm with centrally situated deeply staining round nuclei. The lumen of the midgut in the anterior and the posterior region is wider while in the middle region it is much narrower. The nuclei are large and granular in the anterior region of the midgut while in the posterior region they are smaller and more deeply stain. The longitudinal folds of the epithelium of the posterior region project into the lumen possibly to increase the surface area for the activities of midgut epithelium. Numerous regenerative cells are scattered singly or in groups (nidi) near the bases of the epithelial cells. In the anterior region the regenerative cells are scattered singly but in the posterior region they are in groups of three or four so as to form the nidi. Several globular protrusions are visible arising from the lumen ends of the midgut epithelial cells which are at a distance from the regenerative cells or the nidi. These globular protrusions, which seem to be cell disintegration products, have hitherto been described by Bordas (1920) in adult Lepidoptera and several others in other insects as the secretion vesicles. They are abundant in the older insects.

A very fine peritrophic membrane is perceptible under high magnification in the anterior region of the midgut. It appears that the capacity to secrete peritrophic membrane is localised to the midgut cells of the anterior region only.

The musculature enveloping the midgut epithelium is quite prominent and the layer of circular muscles surround the longitudinal ones.

## THE HINDGUT

The hindgut is the longest part of the alimentary canal and is divisible into two regions viz., the intestine and the rectum. Their walls are histologically similar to that of the foregut but the epithelial cells are large and the longitudinal muscles surround the circular ones throughout.

*The Intestine.* It lies coiled into the posterior abdominal segments and is in the form of a very narrow and tubular duct.

The cuboidal epithelium (Fig.6) is thrown into feeble longitudinal folds. The intima is fairly thick. The cytoplasm of the epithelial cells is alveolar and their nuclei are deeply staining, round and quite large. The basement membrane is very thin and barely perceptible.

*The Rectum.* It is a large wedge shaped structure and continues posteriorly to open to the exterior. Its posterior part tapers more gradually than the anterior one. After the emergence, the rectal contents become gradually excreted to the outside of the body thus the rectum gets very much shrunken in appearance.

The epithelium (Fig. 7) is flat, deeply staining, and small round nuclei are regularly distributed. The cell walls are not seen. The rectal glands which have been described in many moths and butterflies (Bordas 1920), are not present in this insect. Musculature is similar to that of the intestine.

## DISCUSSION

On the basis of his work on the alimentary canal of a large number of Lepidopterous larvae and adults Bordas (1911, 1920) strongly supported Van Gehuchten's (1890) conviction that the globular protrusions seen at the distal ends of the midgut cells are secretion vesicles. Their contention was later supported by Shinoda (1927) who studied the process of secretion in several orders of insects including Lepidoptera. Basing his observations on the study of the postembryonic development of the larval midgut in *Vanessa*, Henson (1929) gave strong evidence to show that these vesicles which have been regarded by previous workers to be responsible for secretion are nothing more than cell disintegration products. Almost simultaneously Yung Tai (1924) studied the development of the alimentary canal in *Gallaria* and regarded these globules to be cytoplasmic disintegration products. Pyle (1940) in his study of the anatomy and histology of the alimentary canal in the moth *Callosomia promathea* did not mention anything about the process of secretion. In *Leucinodes orbonalis* it has been shown that the globular protrusions appear at the distal ends of full sized and old midgut cells. In a newly emerged imago they are either absent or very few in number while in the case of the older individuals their number is large. It is a well known fact, that the moths feed more actively soon after issuing from the pupal cocoon. Therefore, it is unlikely that these globules are related to secretory processes, because if we regard them to be secretory their production should have been more when the insect was feeding actively and vice versa. Observations in the insect under study show that they are either absent or very few in number in the midgut of young imagos. Besides, these globules are observed to arise invariably from the distal ends of the older cells which are further apart from the regenerative cells and never arise from the cells surrounding the regenerative nidi. Hence, in view of the above considerations, it is reasonable to believe that these globules are not in any way related

to secretion processes and really represent the cell disintegration products. It further appears that the secretion is always in the form of diffusible liquids from the entire surface of midgut epithelium.

Another important fact revealed by the present investigation is the presence of a thin peritrophic membrane in the midgut of the imago. The peritrophic membrane has long been regarded to be absent from the midgut of adult Lepidoptera. Wigglesworth (1950) stated that the function of peritrophic membrane is to protect the delicate midgut epithelium from the hard particles of food and that it performs the function of mucus in the vertebrates, a substance which is absent from the insect midgut. In support of his contention he added that it is absent in most of the fluid feeding insects e.g., adult Lepidoptera, Hemiptera and some Diptera. It was felt that the foregut and the hindgut remain safe from injuries which may be caused by hard particles of food because of being provided with a chitinous intima lining the epithelium. Aubertot (1938) was the first worker to report the presence of a peritrophic membrane in the imaginal midgut of *P. brassicae*. But their belief in the absence of peritrophic membrane was, rather, so firmly held that his observation was not attached much importance. Recently, however, Waterhouse (1953) showed that the peritrophic membrane occurs in many such insects where it is generally believed to be absent, for example, in many adult of Lepidoptera, Namatocera and Orthorrhapha (Diptera), in larval Dermestidae and adult Carabidae (Coleoptera), certain adult ants (Hymenoptera) and in Gryllotalpa (Orthoptera). Thus he showed that the peritrophic membrane is present in many such insects which have no occasion to protect the midgut epithelium from hard particles of food. In the adult of *L. orbonalis* peritrophic membrane has been detected by the author in the anterior region. It seems that the capacity to secrete the peritrophic membrane is localized in that region only. It is difficult to suggest any function for the peritrophic membrane for such insects which feed on fluid diet. But its presence in the fluid feeding insects disapproves the view that its function is to protect the midgut epithelium and that it has evolved independently in those insects in which it was needed. In fact, since in most of the primitive insects e.g., Collembola, Thysanura, Mecoptera, etc, a peritrophic membrane is present. It is clear that its absence in certain groups and individuals is due to its disappearance in course of evolution.

#### SUMMARY

The anatomy and histology of the alimentary canal of the adult *Leucinodes orbonalis* Guen. has been described in detail. The foregut is divided into four regions, viz., pharynx, oesophagus, crop and the oesophageal valve. It has been shown that the globular protrusions found arising from the distal ends of the midgut epithelium are nothing more than cell disintegration products. A thin peritrophic membrane has been reported to be present in the anterior region of the midgut and its significance has been discussed. The hindgut has been divided into the intestine and the rectum. Rectal glands have been shown to be absent in this Pyraustid.

#### ACKNOWLEDGEMENT

The work was carried out in the Zoological Research Laboratories of Allahabad University. The author is extremely grateful to Mr. S.C. Verma and Dr. U. S. Srivastava for taking keen interest in the supervision of this work. Thanks are also due to the authorities of the University and the Government of India for the award of the Senior Research Scholarship and to the Head of the Zoology Department for providing the necessary laboratory facilities.

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